BLOOD-STAGE DYNAMICS AND CLINICAL IMPLICATIONS OF MIXED 
PLASMODIUM VIVAX–PLASMODIUM FALCIPARUM INFECTIONS

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Abstract. We present a mathematical model of the blood-stage dynamics of mixed Plasmodium vivax–Plasmodium falciparum malaria infections in humans. The model reproduces features of such infections found in nature and suggests several phenomena that may merit clinical attention, including the potential recrudescence of a long-standing, low-level P. falciparum infection following a P. vivax infection or relapse and the capacity of an existing P. vivax infection to reduce the peak parasitemia of a P. falciparum superinfection. We simulate the administration of antimalarial drugs, and illustrate some potential complications in treating mixed-species malaria infections. Notably, our model indicates that when a mixed-species infection is misdiagnosed as a single-species P. vivax infection, treatment for P. vivax can lead to a surge in P. falciparum parasitemia.

Plasmodium vivax and P. falciparum are the most widespread and commonly studied of the four species that cause human malaria. Dual infections are common and frequently recorded in field surveys, but there has been little research on the within-host interactions or clinical impacts of coinfecting species. Cohen1 reviewed prevalence surveys and concluded that in general, fewer mixed-species infections are observed than would be expected from the prevalences of the constituent species. Richie2 reviewed prevalence surveys and concluded that there is no general pattern in the frequencies of mixed-species infections in humans. Our reviews of more recent cross-sectional studies3,4 found that lower-than-expected frequencies of dual P. vivax–P. falciparum infections correspond to higher overall malaria prevalence, and, in general, that the frequencies of mixed-species Plasmodium infections detected in humans may depend upon the particular combinations of Plasmodium species present. We have also found mixed-species Plasmodium infections common in vector Anopheles species.5

Longitudinal studies of mixed-species infections are extremely rare. Several neurosyphilis malariatherapy charts published by Boyd and Kitchen6,7 suggest that P. falciparum suppressed P. vivax parasitemia in these patients. Interspecies inhibition was also suggested by other such studies.8–11 Shute12 reported that P. vivax often failed to thrive if inoculated simultaneously with P. falciparum, but could reach patent levels if inoculated a few days before P. falciparum. More recently, clinical studies13,14 have found high rates of P. vivax infection following treatment of patients previously assumed to be infected only with P. falciparum. Studies with non-human malarialas have also suggested interspecific suppression.15–19

The pathologic consequences of mixed-species infections are of particular interest to clinicians. Jeffrey20 noted that prior infection with P. ovale could alter the clinical course of subsequent P. falciparum infection. Other studies have noted relationships between mixed-species infections and enlarged spleen size,20 decreased spleen size,21 and depressed immune response.22 Black and others23 suggested that P. malariae infections reduce the severity of subsequent P. falciparum infections, and that individuals concurrently infected with both species have fewer clinical symptoms than those infected with P. falciparum alone. Similar conclusions have been reported for P. vivax and P. falciparum.23–26, but see 27,28

The work of Black and others inspired us to develop a mathematical model of P. malariae–P. falciparum within-host dynamics.29 With that model, in addition to reproducing known features of those infections, we found that an existing P. malariae infection can reduce the peak parasitemia of a subsequent P. falciparum superinfection by as much as 50%. Here we address the dynamics of a more common and clinically significant mixed-species malaria infection—one with P. vivax and P. falciparum—and investigate several of its clinically important features.

METHODS

We adapt our model of mixed P. malariae–P. falciparum infections30 to the more clinically common dual infection with P. vivax and P. falciparum. Unless stated otherwise, the structure and assumptions of the adapted model are as described in detail elsewhere. Briefly, the model is a set of five nonlinear ordinary differential equations:

\[ \frac{dV}{dt} = aV - cJV - c_xJV - c_yJV - gV, \]
\[ \frac{dF}{dt} = bF - cKF - c_yF - c_yF - gF, \]
\[ \frac{dI}{dt} = s_x(V + F) - q_xI, \]
\[ \frac{dJ}{dt} = s_yV - q_xJ, \]
\[ \frac{dK}{dt} = s_yF - q_xK. \]

The dynamic variables V and F represent per-microliter densities of P. vivax and P. falciparum asexual forms, respectively. I represents the per-microliter density of the effectors of the non-specific immune response, and J and K represent those of the effectors of the species-specific immune responses raised by V and F, respectively.

a and b represent the asexual-form replication rates for P. vivax and P. falciparum, respectively. Although the P. vivax multiplication rate may vary from 12 to 24 merozoites per merozoite,30 we follow the more recent authority of Garnham31 and assume that an average P. vivax merozoite produces 13 merozoites every 2 days. If considered as a continuous (asynchronous) process of exponential growth, this fixes \( a = (\ln 13)/2 = 1.28 \). If an average P. falciparum mer-
ozone produces 16 merozoites every 2 days,\textsuperscript{32} \( b = (\ln 16)/2 = 1.39 \).

\( g \) is the gametocyte conversion rate, at which \( P. \) \textit{vivax} and \( P. \) \textit{falciparum} asexual forms differentiate to sexual, transmissible forms. In previous work\textsuperscript{33,34} we examined a range of values for \( g \) consistent with the rates observed for \( P. \) \textit{falciparum}.\textsuperscript{35,36} Given our observations in those studies, and because there is not sufficient empirical evidence to suggest otherwise, here we adopt \( g = 0.04 \) for both \( P. \) \textit{vivax} and \( P. \) \textit{falciparum}.

\( q_s \) is the specific-immunity decay rate, at which the specific immune effectors senesce. Specific immune effector life-spans vary from hours for cytokines to years for memory B cells.\textsuperscript{37} Here we follow our previous convention\textsuperscript{33,38} and arbitrarily set \( q_s = 0.01 \), implying an effector half-life of 70 days.

\( q_n \) is the non-specific-immunity deactivation rate at which the non-specific immune effectors become quiescent. Macrophages are longer-lived than B or T cells;\textsuperscript{37} thus, for the time-scale of this system, we are more concerned with the deactivation than the senescence of macrophages. Antia and Koella\textsuperscript{39} approximate the half-life of activated macrophages at one day; for convenience we set \( q_n = (\ln 2)/1.1 = 0.6 \), implying a half-life of 1.1 days.

\( c_s \) and \( c_n \) are the specific and non-specific capture/removal rates, respectively, at which the specific and non-specific immune effectors eliminate parasites from the circulation. \( s_s \) and \( s_n \) are the specific- and non-specific-immunity proliferation rates, respectively, at which the specific and non-specific immune effectors are generated. Since \( c_s, c_n, s_s, \) and \( s_n \) are mathematical abstractions rather than known biological rates, we examined model output over a wide range of values, varying each of these parameters from 0.0001 to 1,000 by orders of magnitude.

\( x \) and \( y \) correspond biologically to the degree to which epitopes are shared between species, and thus to the ability of specific immune effectors generated against one species to cross-react with the other species. We assume here that the degree of specific-immunity cross-reactivity translates directly into the rate of parasite removal, and that \( 0 \leq x, y < 1 \) (since it is unlikely that effectors generated against one parasite would recognize another parasite with higher affinity). Although elsewhere\textsuperscript{29} we explored conditions under which \( x \neq y \), here we assume \( x = y \). Table 1 summarizes the variables and parameters considered in the model.

The model was analyzed using two basic approaches. Equilibria were determined and analyzed using Mathematica 3.0 (Wolfram Research, Champaign IL); analytic solutions for \( P. \) \textit{vivax}-\textit{P. falciparum} infections are identical to those described elsewhere,\textsuperscript{29} except for the substitution of \( V \) for \( M \). To observe the dynamics of the model over time, we integrated the system of equations, using fourth-order Runge-Kutta methods in the C programming language, with time intervals of 1/32 day over 365 days, and tested its behavior over a wide range of parameter space. For computer runs, we set initial merozoite densities at 0.01 parasites/\( l \) of blood and initial \( I, J, \) and \( K \) densities at 0.00001/\( l \). Super-infection was approximated by maintaining the parasite density of the delayed parasite = 0 until a pre-determined time-delay was reached. Runs in which the parasitemia of either or both species exceeded 1,000,000/\( l \) were considered fatal and discarded from analyses.

The administration of antimalarials was approximated by assuming a parasite killing rate consistent with published ranges of the parasite reduction ratios (PRRs, the fraction of parasites reduced per asexual life cycle).\textsuperscript{41} Since parasite growth was assumed to be asynchronous, a continuous PRR (\( k \)) was calculated from published values based on a 48-hr observation. To investigate conditions of drug-sensitive and drug-resistant \( P. \) \textit{falciparum}, we tested treatment of mixed infections with both (I) quinine (to treat \( P. \) \textit{falciparum}) and (II) a mefloquine/primaquine combination (to treat \( P. \) \textit{vivax}) in areas of \( P. \) \textit{vivax} chloroquine resistance, or \( P. \) \textit{vivax} cases with suspected mixed infection with chloroquine-resistant \( P. \) \textit{falciparum}). For both quinine and mefloquine, PRR = 10–10\textsuperscript{5},\textsuperscript{41} for PRR = 10, \( k = -1.45 \); PRR = 100, \( k = -2.30 \); PRR = 1,000, \( k = -3.45 \). Drug resistance was approximated by restricting drug action on the resistant parasite to the period of time before antimalarial (in this case, mefloquine) concentration decreased below the minimum parasitidal

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concentration (MPC). We followed the example of White and used $MPC_{\text{resistant}} = 1,000 \text{ ng/ml}$ of serum. Thus, given an initial serum concentration of mefloquine of $2,000 \text{ ng/ml}$, mefloquine will remain active for approximately 14 days, equivalent to its terminal elimination half-life.\(^{42}\) *Plasmodium vivax* relapse was approximated by resetting $V = 0.01$ at 16 days following quinine treatment and at 41 days following mefloquine/ primaquine treatment, as extrapolated from the time at which *P. vivax* appears in the blood following treatment.\(^{14}\)

**RESULTS**

**Equilibria.** Equilibrium analyses are presented in detail elsewhere.\(^{29}\) As in mixed *P. malariae*- *P. falciparum* infections, there are 4 stable equilibria ($V^*, F^*, I^*, J^*$, $K^*$) for this system of equations: 1) the trivial equilibrium, at which all parasites have been eliminated and no immune effectors are present; 2) an equilibrium at which *P. falciparum* has been eliminated by the immune response and only *P. vivax* remains in the blood; 3) an equilibrium at which *P. vivax* has been eliminated by the immune response and only *P. falciparum* remains in the blood; and 4) an equilibrium at which both species coexist in the host. The equilibrium asexual-immune-response rate coefficients, $(F^*)$ can be readily expressed as functions of two products of immune-response rate coefficients, $(c_s s_n)$ and $(c_n s_s)$.

For species coexistence (equilibrium 4), with the parameter values given above, it is necessary that for $V^* > 0$, $c_s s_n/c_n s_s < 676.4 - 736.4 x$ and for $F^* > 0$, $c_n s_n/c_s s_s > 676.4 y - 736.4$. Since $y$ is always $< 1$, the second statement is always true, and species coexistence depends on whether the conditions of the first statement are met; note that $x < 0.9185$ (as $c_s s_n, c_n$, and $s_s$ are $> 0$). Most patterns of system equilibria closely resemble those in the *P. malariae*- *P. falciparum* model, but with the important difference that *P. vivax* can persist in coinfections at values of the ratio $(c_s s_n)/(c_n s_s)$ that are up to 11 times greater than the maximum values at which *P. malariae* can persist. This capacity of *P. vivax* to persist under conditions of higher non-specific immunity indicates the importance of asexual replication rates in determining species survival within mixed-species infections.\(^{29}\)

**Dynamics: simultaneous infection.** The intuitive nature of the 4 equilibria belies the complex patterns of dynamics that precede system equilibration. Under the assumptions noted above, when both species persist in the blood (i.e., when the conditions for equilibrium 4 are met), simultaneously-initiated infections exhibit several similarities over the entire range of tested parameters. First, following their initial appearance in the blood, both the *P. falciparum* and *P. vivax* populations reach maximum peaks within 13–24 days. For each species, this initial peak represents its highest asexual-form density of the infection, and for each it is followed by a sharp decrease to a parasitemia as much as 5 orders of magnitude below the initial peak. Peak heights follow the patterns described elsewhere.\(^{29}\)

Following the initial peak, the behavior of the system becomes quite complicated and varies with parameter values. If specific immunity is greater than non-specific immunity, i.e., if $c_s s_n > c_n s_s$, the *P. vivax* and *P. falciparum* populations generally synchronize, entering cyclical patterns in which their peak values appear at approximately identical time points during the course of the infection (Figure 1A). If $c_s s_n < c_n s_s$, the species densities oscillate out-of-phase (Figure 1B). Increasing the cross-reactivities of specific immune responses (i.e., increasing $x$ and $y$) generally dampens oscillations, lowering the peak—since each parasite must overcome a stronger immune response—and raising the trough parasitemias. Higher cross-reactivities also reduce the frequencies of oscillation of *P. falciparum* and *P. vivax* densities, since species succession is slower when the suppressed parasite must overcome cross-reactive specific immune effectors generated by the peak parasite. A comparison of *P. vivax*- *P. falciparum* and *P. malariae*- *P. falciparum* mixed infections indicates that greater values of $a$ (the asexual form replication rate; $P. vivax > P. malariae$) produce both higher secondary peaks and higher oscillation frequencies of the coinfecting species.

**Dynamics: superinfection.** Parasite dynamics following a superinfection of one species by the other vary with the timing of the superinfection. If *P. vivax* superinfects *P. falciparum* early in the infection, appearing in the blood during the period of an initial peak *P. falciparum* parasitemia (1 to 13–24 days), peak *P. vivax* parasitemia is lower than its peak when the species appear in the blood simultaneously. In contrast, if *P. vivax* superinfects a *P. falciparum* infection that has equilibrated at a lower density, *P. vivax* attains a higher peak parasitemia than under conditions of simultaneous ap-
pearance, and produces a secondary peak in *P. falciparum* density (Figure 2). The same phenomenon occurs when *P. falciparum* superinfects a low-level *P. vivax* infection. Finally, if *P. vivax* appears in the blood at least 1–2 days before *P. falciparum*, it can attain a peak parasitemia up to 4 times higher than when the species appear simultaneously, and can reduce the peak *P. falciparum* parasitemia by up to 28%. An example is given in Figure 3.

Note that the pre-erythrocytic stage of *P. falciparum* lasts 5.5–7 days, and that of *P. vivax* 6–8 days. Thus, on average, *P. vivax* sporozoites must be inoculated approximately a day before *P. falciparum* if the 2 are to appear simultaneously in the blood. Thus, all values refer to simultaneous blood appearance, not inoculation; simultaneous inoculation was approximated by delaying the appearance of *P. vivax* in the blood by 0.75 days.

**Dynamics: drug treatment.** Antimalarial treatment led to rapid decreases in parasitemia; as expected, the parasite clearance time was directly related to parasitemia at the time of antimalarial administration. Treatment with quinine led to elimination of both parasites, followed later by a relapse of *P. vivax*, first patent approximately 21 days after quinine administration (see Methods). In cases with mefloquine-resistant *P. falciparum*, treatment with mefloquine/primaquine led to the elimination of *P. vivax* and a rapid decrease in *P. falciparum*, followed by a *P. falciparum* recrudescence that began approximately 14 days after drug administration, but failed to reach patency (>10 parasites/µl) for up to 40 days thereafter. Under conditions of both relapse and recrudescence, the surviving parasite exhibited a resurgence in parasitemia, to levels higher than those immediately preceding drug administration. Under conditions of low specific, and high non-specific immunity, this second peak was occasionally found to surpass the initial peak, although it was not possible to obtain the exact range of values at which this phenomenon occurs. Figure 4 shows examples of treatment with quinine and mefloquine/primaquine.
time-lag. Thus, when \( P. vivax \) density eventually decreases, and \( P. falciparum \) recrudesces, there is a lower \( P. falciparum \)-specific-immune response present to suppress it; consequently, it attains a higher parasitemia than that immediately before the \( P. vivax \) superinfection. This possibility is particularly important in mixed infections involving \( P. vivax \), since \( P. vivax \) can reappear in the blood following either a new inoculation or a relapse from liver hypnozoites; the potential for a \( P. falciparum \) resurgence triggered by a \( P. vivax \) relapse indicates an unexpected hazard of malaria infections that might be considered benign. Stable low-level \( P. falciparum \) parasitemia may suggest the familiar concept of pre-immunity; our model indicates that a disruption might lead to a resurgence in parasitemia, perhaps with clinical consequences.

\( Plasmodium malariae \) superinfection can produce the same general effect, although it is much less pronounced. Due to the greater asexual-form multiplication rate of \( P. vivax \), immunity raised by \( P. vivax \) causes \( P. falciparum \) density to decrease further than does \( P. malariae \) in a comparable mixed infection, but, as a consequence (due to the decrease in specific immunity), to increase to a higher subsequent peak. An interesting practical consequence is that in addition to the other challenges of detecting and differentiating the species, the likelihood of detecting \( P. falciparum \) in a mixed \( P. falciparum\)-\( P. malariae \) infection may be reduced, and the prevalence of mixed infections under-reported. More generally, the likelihood of detecting any of the species in a mixed-species infection may depend not only on methodology and the characteristics of each species, but on the relative timing of inoculation with each species.

In contrast to the deleterious side effects of \( P. vivax \) superinfection, we found that an existing \( P. vivax \) infection could substantially reduce the peak parasitemia of a \( P. falciparum \) superinfection, provided that \( P. vivax \) appeared in the blood at least 1–2 days before \( P. falciparum \). This parallels our findings with mixed \( P. malariae\)-\( P. falciparum \) infections: if \( P. malariae \) entered the blood at least 12 days before \( P. falciparum \), its peak parasitemia was up to 500 times higher than that of simultaneous appearance, and peak \( P. falciparum \) levels were reduced by up to 50%.

Many factors confound the relationship between parasitemia and disease, but there is generally a loose positive correlation between circulating parasite load and clinical status. It seems likely that one primary factor that lowers the correlation is that only a fraction of \( P. falciparum \) parasites appear in the peripheral circulation, and that total parasite load is a poor measure of infection intensity.
density (as considered in our model) is more closely correlated. Thus, overall our model suggests that 
*P. vivax*-*P. falciparum* interactions in mixed infections can have profound clinical effects in both uncomplicated malaria (perhaps by maintaining 
*P. falciparum* densities below fever threshold) and severe cases. This suggestion is supported by the clinical findings of Luxemburger and others, who reported that severe malaria was 4.2 times more common in patients with only 
*P. falciparum* infections than in those with mixed 
*P. falciparum*-*P. vivax* infections. Maitland and others have proposed that the protective potential of 
*P. vivax* may be so strong that 
\(\alpha\)-thalassemias are positively selected in a population by predisposing individuals to 
*P. vivax* infections and thereby protecting them against 
*P. falciparum*. Our model indicates that the interspecific interactions may be quite complicated, with the timing of the species infections determining whether 
*P. vivax* ameliorates or exacerbates subsequent 
*P. falciparum* infection.

Perhaps the most intriguing results for clinicians concern drug treatment. In our model, the interval from drug treatment to 
*P. falciparum* recrudescence is generally longer than to 
*P. vivax* relapse; treatment usually diminishes 
*P. falciparum* parasitemia to a level below that of an initial post-hepatic merozoite cohort, and thus it takes longer to recrudescence to patent levels. This also suggests that some 
*P. vivax* relapses might actually be recrudescences, especially in drug-resistant cases. Numerous reports have documented 
*P. vivax* relapse following drug treatment for what was presumed to be a single-species 
*P. falciparum* infection. The interval between treatment and relapse depends on the half-life of the drug administered. For example, Loaoresuswan and others found that 33–40% of patients treated with different anti-
*P. falciparum* artemether regimens exhibited 
*P. vivax* relapse within a 28-day period, while none of those treated with mefloquine (with a half-life nearly 30 times that of quinine) relapsed during the same follow-up period. More importantly, if one of the parasites in a mixed infection is resistant to the antimalarial, it may surge in density after the other parasite is removed (Figure 4).

The possibility that recrudescence may follow drug treatment emphasizes the clinical importance of accurate, specific diagnoses. Recent studies have pointed to highly relevant limitations of traditional microscopy-based detection techniques. For example, through acridine-orange staining, nested polymerase chain reaction, and microtiter-plate hybridization techniques, Zhou and others demonstrated that the proportion of infections diagnosed in patients on the Thailand-Myanmar border by Giemsastain microscopy as single-species 
*P. falciparum* and two-thirds of those diagnosed as single-species 
*P. vivax* were mixed-species infections. Our model suggests that such diagnostic discrepancies may have severe clinical consequences.

Although our model incorporates human immune responses as the critical media of parasite interaction, we have neglected a number of complexities that merit further attention. First, 
*P. vivax* might reduce 
*P. falciparum* parasitemia by initiating fever. Indeed, the pyrogenic threshold of 
*P. vivax* is much lower than that of 
*P. falciparum* (150–200 versus 1,500–10,000 parasites/ul). In our model, even under conditions of simultaneous appearance in the blood, in which 
*P. falciparum* growth greatly exceeded that of 
*P. vivax*, 
*P. vivax* usually reached its pyrogenic density first. Second, since 
*P. malariae* prefers mature erythrocytes and 
*P. falciparum* appears to prefer younger cells, in our previous model we did not consider red blood cell competition as an important factor. However, since 
*P. vivax* selectively invades reticulocytes, red blood cell competition (as well as 
*P. falciparum*-induced dyserythropoiesis) may be an important factor in mixed 
*P. vivax*- 
*P. falciparum* infections, as may interspecific competition for nutrients in the bloodstream. Third, if interspecific cross-immunity does exist, it is possible that it is anti-immune rather than anti-parasite. Our study does not eliminate the possibility of an anti-immune cross-protection, but suggests that it is possible for protection to be gained by anti-parasite effects alone. Fourth, continuous parasite growth is only an approximation; 
*P. vivax* in particular can be highly synchronous. An integration of our model with models of parasite synchronization, and of antigenic variation would be of great interest. Finally, although we have focussed on the clinically important aspects of mixed infections, we have focussed on asexual blood forms and have not explicitly considered gametocytes, the dynamics of which are clearly critical to species interactions, including their geographic and seasonal distributions.

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